5. The anti-anæmic liver principle is not identical with, and occurs independently of, the methæmoglobin-producing factor, which is present in about 15–20 p.c. of liver extracts irrespective of their hæmopoietic potencies.

6. The methæmoglobin test of Duesberg and Koll gives no indication of the presence or absence of anti-anæmic potency in liver extracts used for the treatment of pernicious anæmia, and the clinical test still remains the only reliable method of assay.

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FURTHER EVIDENCE THAT MAMMALS CANNOT ACCLIMATIZE TO 10 P.C. OXYGEN OR 20,000 FEET ALTITUDE.

J. ARGYLL CAMPBELL.

From the National Institute for Medical Research, Hampstead.

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IN earlier communications (Campbell, 1927*a*, *b*; 1928; 1930) evidence was presented indicating that mammals cannot acclimatize fully to oxygen at 10 p.c. or less of an atmosphere, equivalent to an altitude of 20,000 ft. The results recorded in the present paper indicate that while young mice and rabbits can grow at normal rate under 12 p.c. oxygen (equivalent to 18,000 ft. altitude) they cannot do so under 10 p.c.; under the latter they deteriorate progressively and die.

It must be emphasized that we are dealing with continuous and prolonged exposure to oxygen deficiency; it is well known that some mammals will tolerate even 4 or 5 p.c. oxygen for short periods without any previous acclimatization, but they are more or less distressed or collapsed. By acclimatization is meant maintenance of normal appetite leading to normal health, vigour, metabolism and body-weight. As already recorded in the papers referred to above, rabbits and mice appear to have greater powers of resistance to low oxygen pressure for prolonged periods than has man. Therefore if these animals cannot tolerate 10 p.c. oxygen continually, it is not likely that man will do so.

EXPERIMENTS WITH MICE.

Methods.

The mice were enclosed in respiratory chambers—described in the earlier papers—up to about 600 litres capacity, the air within being well mixed by means of a fan driven by a motor outside the chamber. Oxygen used was replaced automatically from a spirometer, while the carbon dioxide and water vapour produced were absorbed by soda-lime spread out in trays near the top of the chamber. The oxygen percentage in the chamber was lowered by the addition of the requisite quantity of nitrogen, and daily analyses were carried out to be sure that the oxygen percentage was at the desired level; it was not allowed to vary more than ½ p.c. above or below this. It has been proved that lowering the oxygen pressure by diluting the air with nitrogen has a similar effect upon animals to that of lowering the barometric pressure. Sufficient food, bedding and water were placed in the boxes to cover four days at a time, so that the chambers were opened for about twenty minutes only every three or four days. The temperature of the air was kept at about 20° C.

Two diets were employed which, under normal conditions, give much the same rate of growth during the 2nd to 8th month period, the stage covered by the mice in the present Experiments A and B. In Experiment A the diet consisted of a mixture of oats, barley and wheat with a little clean green-stuff. In Experiment B the special diet for breeding mice at this Institute was used; it contained brown bread—5 days old—soaked in milk, best white oats, and on occasion rolled oats, wheat germ, millet, hemp, canary seed, linseed, sprouted oats and cod-liver oil.

The mice used were all about 2 to 3 months old at the start and weighed from 16 to 20 g. The main details of the experiments are given in Table I, which contains also the summary of the previous experiments with mice (2).

TABLE I.

Experiments.	Number of mice.	Number of days to reach.		Number of days at 10 p.c. O_2—	
		12 p.c. O_2.	10 p.c. O_2.	To cause loss of weight.	To cause marked increase in death-rate.
Previous (2)	24	7	14	<7	14–28
Present, A	75	14	28	<7	28
Present, B	54	14	64	<7	35

In Experiment A of the present series 75 mice were used both for exposure to low oxygen pressure and for the controls, while in Experiment B 54 mice were employed in each case.

Loss of Body Weight.

In the previous experiments the oxygen pressure was lowered gradually from 20·9 p.c. to reach 12 p.c. on the 7th day ; it was kept at this level for another 7 days before being lowered to 10 p.c. on the 14th day (Table I). In Experiments A and B, 14 days were taken to reach 12 p.c. oxygen ; and another 14 days elapsed in Experiment A before the pressure was lowered

FIG. 1.

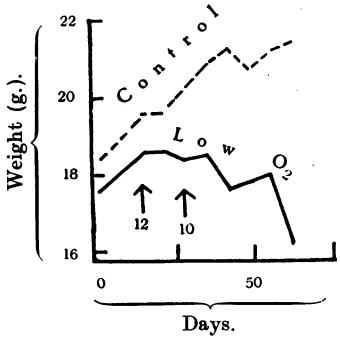

FIG. 2.

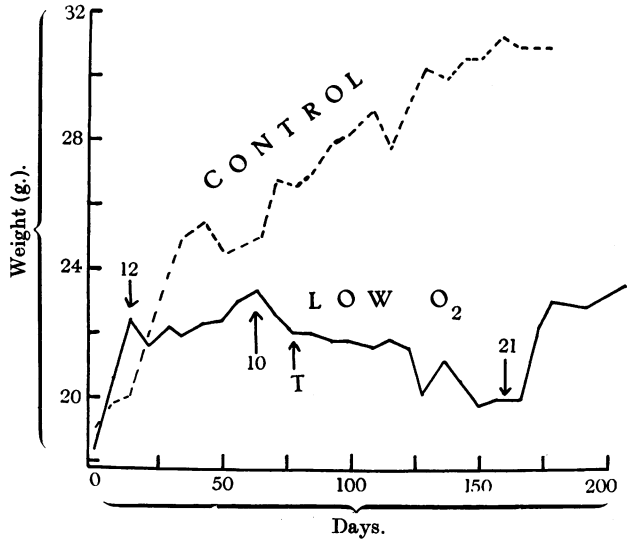

FIG. 1.—Body-weight changes of mice in Experiment A. The figures near the arrows refer to the oxygen pressure.

FIG. 2.—Body-weight changes of mice in Experiment B. The figures near the arrows refer to the oxygen pressure. T = first appearance of tetany.

from 12 to 10 p.c. ; while in Experiment B, 50 days were spent at the 12 p.c. level. Thus in previous experiments only about 14 days were spent in acclimatization at oxygen levels higher than 10 p.c., but 28 days were allowed in Experiment A and 64 days in Experiment B. Although this was so, yet in all three experiments the mice showed loss of weight and weakness during the first week at 10 p.c. level (Table I), and thereafter the decline in weight and vigour was progressive. In the previous experiments the surviving mice lost 5 g. each in 4 to 5 weeks under 10 p.c. oxygen, while the controls gained 3·5 g. (2). In Experiment A (Fig. 1) they lost on an average 3 g. in 6 weeks, while the controls gained 3 g. in the same time. In Experiment B (Fig. 2) the mice lost on an average 3 g. in 8 weeks, while the controls gained 6 g. With the extra acclimatization at 12 p.c. oxygen

the mice in the present Experiments A and B lost weight more slowly than the mice in the previous experiment; that is, the extra acclimatization delayed the progressive loss of weight somewhat, but could not check it completely. In Fig. 2 it will be observed that about the 30th day of the experiment the mice began to grow again under 12 p.c. oxygen; this is definite evidence that they were acclimatized to 12 p.c. before being exposed to 10 p.c.

The experiments were allowed to continue under 10 p.c. oxygen until it was obvious that acclimatization of the survivors was impossible. This occurred on the 69th day from the start of Experiment A (Fig. 1), and on the 159th day of Experiment B (Fig. 2). Then the oxygen was raised again to the normal level of about 21 p.c. Some of the survivors began to grow again under these conditions (Fig. 2), but others did not, and died later (Fig. 3).

FIG. 3.

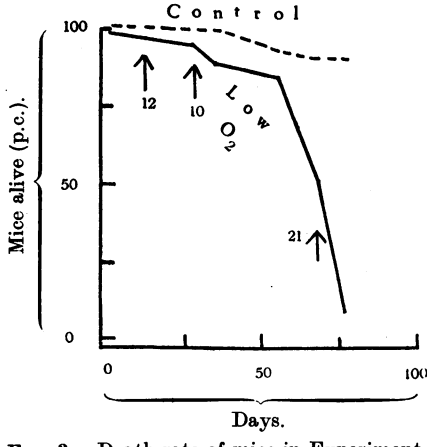

FIG. 3.—Death-rate of mice in Experiment A.

FIG. 4.

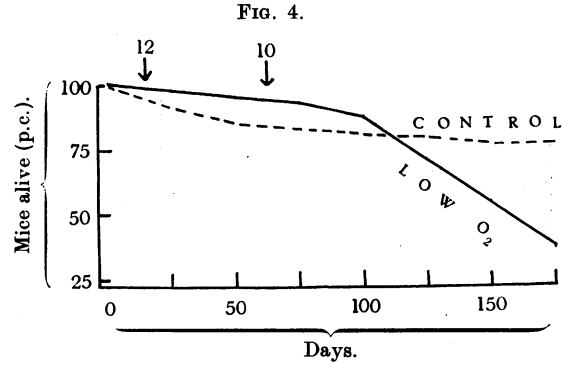

FIG. 4.—Death-rate of mice in Experiment B.

Death-Rate.

In Experiments A and B the death-rate under 10 p.c. oxygen in about 4–5 weeks' time greatly exceeded that for the controls (see Figs. 3 and 4). In Experiment B (Fig. 4) loss of life was not so rapid in rate as in Experiment A (Fig. 3). This was evidently due to the longer period of acclimatization allowed under 12 p.c. in Experiment B. As already stated, the mice in the latter experiment were fully acclimatized to 12 p.c. oxygen since they began to grow under this pressure; this apparently increased their resistance to 10 p.c., but only for a time, and slow deterioration was inevitable under this pressure. The appearance of the majority of the mice was that of invalids when under 10 p.c. oxygen, all showing weakness, and many exhibiting nervous symptoms, *e.g.* tetany.

At the end of the exposure to 10 p.c. oxygen in Experiment A, that is on the 69th day, 38 of the original 75 mice were alive; although the oxygen pressure was then raised to normal level, only 7 of these 38 survivors remained alive after a further 14 days at normal pressure (Fig. 3). It is obvious, then, that

for some of the 38 mice the effects of the exposure to 10 p.c. oxygen had reached such a stage that recovery became impossible even when under normal pressure again.

In Experiment B, on the 159th day, or at the end of the exposure to 10 p.c. oxygen, there were 19 survivors of the original 54 mice (Fig. 4). The healthier 6 of these mice were exposed to normal oxygen pressure at 21 p.c. (see Fig. 2), and they began to grow again. The other 13 of the 19 mice were killed for estimation of serum calcium in connection with tetany.

Tetany.

As reported in the previous researches, excitability—leading even to convulsions on disturbance—and tetany were observed in many of the animals exposed for some time to low oxygen pressure. To endeavour to determine the cause, 5 of the 16 survivors with tetany in Experiment B were fed on 1000 international units of vitamin D per mouse *per diem* for two weeks. This did not cure the tetany, and the serum calcium as estimated by my colleague Mr. T. A. Webster, using Clark and Collip's (1925) method, was 12·5 mg. per 100 c.c. Another 5 mice with tetany were not fed on vitamin D, and showed a similar serum calcium, namely 12·0 mg. per 100 c.c. The serum calcium of 5 of the control mice, that is, mice not exposed to low oxygen pressure, was 10·6 mg. per 100 c.c. We may conclude, then, that tetany was not due to low serum calcium ; it was probably the result of direct action of the low oxygen pressure upon the nerve-cells (Campbell, 1926). Some mice with tetany caused by low oxygen pressure do not get rid of these symptoms when exposed to normal oxygen pressure again, but others do so ; there appears to be a stage beyond which recovery becomes impossible. Some animals reach this stage sooner than others.

Histological examination of the parathyroid and thyroid glands of the mice with tetany did not reveal any marked differences when compared with glands from controls. It was proved by passage of brain emulsion of the mice with tetany that there was no infectious agent present.

Condition of the Heart.

As previously demonstrated (Campbell 1928, 1929), congestion with degeneration of the organs with evidence of chronic heart failure constitute the main findings in post-mortem examinations. Of course all organs must be more or less affected by the low oxygen pressure because of the general weakness, loss of appetite and heart failure.

It has been demonstrated (Campbell, 1932, 1934) that mice acclimatize slowly to 0·25 p.c. carbon monoxide, and in these the heart often becomes hypertrophied (Fig. 5). This is regarded as evidence that the heart undergoes strain due to the resulting low oxygen pressure in the tissues, and to increase in viscosity of blood with increase of red corpuscles. Also when animals succumb to prolonged exposure to carbon monoxide, the post-mortem appearances are those of chronic heart failure. The heart, therefore, plays a main part in acclimatization to carbon monoxide poisoning.

It is well known that exposure to low oxygen pressure causes dilatation of the heart, and acclimatization cannot reasonably be stated to have occurred until this is overcome. The heart controls the circulation, and determines to what level the oxygen pressure in the tissues will fall. Although all organs must be concerned, experience with animals indicates clearly the main importance of the heart-muscle in powers to acclimatize to low oxygen pressure. Some animals collapse and die of heart failure long before others.

In the present Experiment B the hearts of the survivors were weighed and compared with controls as shown in Table II and Fig. 5. As already stated, the mice at the start of the experiment were about 2–3 months old and weighed about 18 g. ; the hearts of such mice weighed 0·100 g. At the end

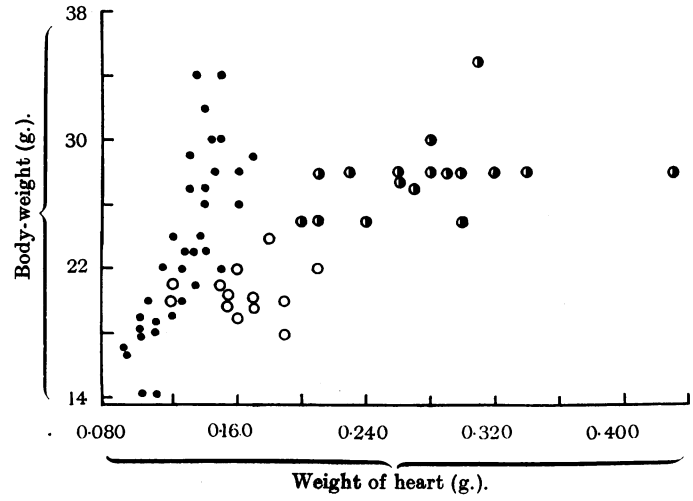

FIG. 5.—Weight of mouse hearts. (*a*) Normal controls, black discs ; (*b*) exposed to 10 p.c. oxygen, clear discs ; (*c*) acclimatized to carbon monoxide, half-black discs.

of the same experiment the mice were about 8–9 months old, and the control mice, that is, those not exposed to low oxygen pressure, weighed on an average 30 g., and their hearts 0·145 g. On the other hand, the survivors of the mice exposed to low oxygen pressure, although also 8–9 months old, weighed only about 20 g., but their hearts averaged more than 0·145 g. (see Table II and Fig. 5). For body-weight and for age some of the hearts of the mice exposed to low oxygen pressure were definitely heavier than those for the controls. This is taken as evidence of attempted hypertrophy, which, perhaps, had occurred chiefly during the 7 weeks under 12 p.c. oxygen pressure ; judging from the growth curve the mice withstood this pressure. The mice in the carbon monoxide experiment were exposed to low oxygen pressure in their tissues for longer periods, namely, 9 months, and were well acclimatized ; some of their hearts show much more hypertrophy. The mice exposed to 10 p.c. oxygen for three months were not acclimatized at all, and thus such hypertrophy could not be expected. Probably if the mice had been exposed

for much longer to 12 p.c. oxygen the hypertrophy would have been more evident. All the hearts recorded in Fig. 5 and Table II were weighed immediately after killing the animals with coal gas, the chambers of the heart being slit open to remove any visible blood. Certainly some blood must remain in some of the capillaries, but this must occur also in the controls. Some deduction should be made to correct for this factor, but in the present case nothing has been deducted; microscopic examination did not indicate that the general conclusions would be affected thereby.

TABLE II.—*Body- and Heart-weights of Mice.*

Numbers and condition of mice.	Body-weight (g.).		Heart-weight (g.).	
	Average.	Limits.	Average.	Limits.
12 controls; 2–3 months old	18	14–20	0·100	0·090–0·120
12 controls; 3–6 months old	23	20–26	0·135	0·115–0·160
11 controls; 8–9 months old; Expt. B	30	27–34	0·145	0·130–0·170
9 low O_2 (10 p.c.); 8–9 months old; with tetany; Expt. B	21	19–24	0·150	0·120–0·180
4 low O_2 (10 p.c.); 8–9 months old; without tetany; Expt. B	20	18–22	0·190	0·170–0·210

Compared with the powers of acclimatization of mice to 0·20–0·30 p.c. carbon monoxide (Campbell, 1934), the resistance to 10 p.c. oxygen seems poor. According to Haldane's (1920; 1922) curves, the blood of a mouse is about 30–35 p.c. saturated with oxygen when breathing 0·25 p.c. carbon monoxide in air, and also when breathing 10 p.c. oxygen, if allowance be made for alveolar air. In both cases the increase in volume breathed and in red corpuscles enables the blood to take up more oxygen than would otherwise be the case. The difference in powers of acclimatization may be connected with the fact that when breathing the carbon monoxide there is 20·9 p.c. oxygen in the air; that is, there is plenty of oxygen in the lungs, although the carbon monoxide prevents the blood taking its full quota. On the other hand, under 10 p.c. oxygen there is a marked deficiency of oxygen in the lungs, and this presents what seems on first thoughts to be a much more difficult task, since there would be less oxygen dissolved in the plasma.

EXPERIMENTS WITH RABBITS.

The animals were enclosed singly in a respiratory chamber of about 150 litres capacity with the same general arrangement as described for the mice. Sufficient hay, oats, green stuff, water and bedding (sawdust) to last about 4 days were placed in the chamber, so that it was opened for only 20 minutes every 3 or 4 days.

In the experiment illustrated in Fig. 6 a young growing rabbit of about 1800 g. was exposed to oxygen pressure gradually lowered from 20·9 p.c. to 12 p.c., reaching this level on the 28th day. The oxygen pressure was maintained at 12 p.c. for another 22 days, and the rabbit's rate of growth under

this pressure was as rapid as is observed in normal rabbits of the same age and weight breathing normal air. The curve for such normal rabbits (cross between Flemish giant and a Belgian hare) has been published elsewhere

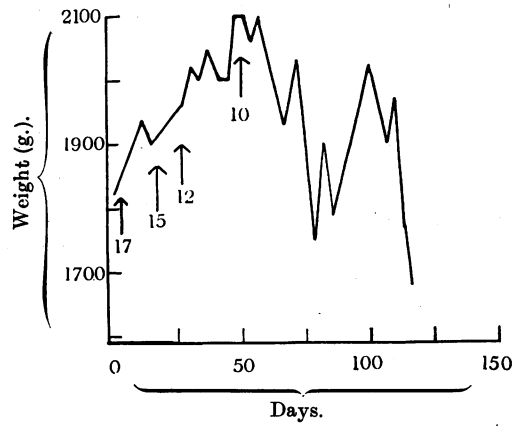

FIG. 6.—Body-weight of rabbit under low oxygen pressure (see figures near arrows).

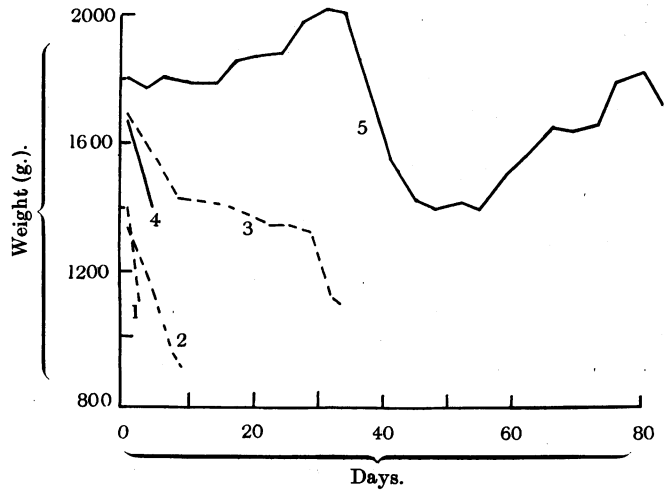

FIG. 7.—Body-weight of 5 rabbits exposed at once to 10 p.c. oxygen. Rabbits 4 and 5 had received previous injections of cobalt chloride and manganese chloride.

(Campbell, 1934), and coincides with that given in Fig. 6 up to the 50th day. It is evident, therefore, that this rabbit had become acclimatized to 12 p.c. oxygen, as judged by rate of growth.

On the 50th day the oxygen pressure was lowered to 10 p.c., when the rabbit lost weight almost immediately, and there was no further increase in

growth above the level of the 50th day. Indeed, the animal lost weight rapidly, though with some apparent attempts to recover again. It was obviously in poor health practically all the time, and eventually died of chronic heart failure on the 118th day, or after 68 days at 10 p.c. oxygen. It had post-mortem appearances characteristic of chronic cardiac failure, *i. e.* dropsy, nutmeg liver, chronic nephritis (large white kidney), marked congestion of organs, dilatation of heart, emphysema of lungs and patches of terminal pneumonia in the lungs. My colleague, Dr. Perdrau, kindly tested by passage the brain and spinal cord for presence of infectious agents, which might explain nervous or other symptoms ; the results were negative.

When compared with the acclimatization exhibited by a rabbit to breathing 0·20–0·30 p.c. carbon monoxide in the air (Campbell, 1934), the resistance when under 10 p.c. oxygen pressure is poor ; as already stated, this difference occurs also with mice.

In Fig. 7 are given the changes in body-weights of 5 young growing rabbits exposed at once to 10 p.c. oxygen without any previous acclimatization to lesser degrees of oxygen deficiency. Rabbits 4 and 5 had their red corpuscles increased by previous daily injections, on about 16 occasions, of solutions containing 7 mg. of cobalt chloride and 3 mg. of manganese chloride (Kleinberg, 1934). In this way the red corpuscles of Rabbit 4 were increased from 4,750,000 to 7,600,000 per c.mm., and of Rabbit 5 from 5,600,000 to 8,555,600, while the hæmoglobin was increased from about 60 to 80 p.c. Rabbits 1, 2 and 3 received no such injections.

The previous increase in red corpuscles does not seem to have aided Rabbit 4 at all, since it died in a few days, but Rabbit 5 seems to have exhibited considerable extra resistance to the effects of breathing 10 p.c. oxygen. It appeared at first to maintain and even increase its weight, but this may have been due to œdema and dropsy, which are often observed *post mortem*. It should have grown regularly, as it was a young growing animal. It certainly looked in poor health, and lost much weight after about 4 weeks under 10 p.c. It recovered somewhat, but was never healthy, and died on the 83rd day. Then it showed all the post-mortem conditions typical of chronic heart failure. Its heart weighed 7·76 g., which is a high figure for its final weight, but not so high perhaps for its age. Controls of the same body-weight have given 4–5 g. as weight of heart and of the same age about 7 g. or less.

Rabbit 3 also exhibited some resistance, and it is interesting that its red corpuscles were not greatly different in number from those for Rabbit 2. Here, again, there is evidence that the hearts of some normal animals resist the low oxygen pressure much more vigorously than those of other apparently normal animals, but all die sooner or later from its effects.

CONCLUSION AND SUMMARY.

From the evidence submitted herein it is obvious that the mammals which have been proved to be most resistant to low oxygen pressure cannot be acclimatized to live in health under 10 p.c. oxygen, equivalent to an altitude of 20,000 ft. Growth ceases, progressive loss of weight sets in, and the animals die sooner or later with symptoms typical of chronic heart failure. There

is evidence that powers of resistance vary greatly in different animals, and depend in great part on response of the heart muscle.

The animals tested grew at normal rate under 12 p.c. oxygen, equivalent to an altitude of 17,000–18,000 ft. There is evidence that Tibetans, yaks and other mammals reside not far from this level at least for some months.

It is not reasonable to speak of true acclimatization above 20,000 ft. Some animals and man exhibit considerable resistance to 10 p.c. oxygen, but the evidence from animal experiment, and in addition that recorded by Major Hingston (1925) and Dr. Raymond Greene (1934), indicate that this is purely a temporary and rather misleading phenomenon. At Everest, Camp III, 21,000 ft., the deterioration was eventually rapid. The resistance in the fittest subject may persist long enough for him to reach the summit of Everest, but the risk is very great from oxygen want alone, without the added exertion and dangers of climbing and exposure to cold.

There is evidence also that much more time should be given to acclimatization below 20,000 ft., that is, at about 12 p.c. oxygen or 18,000 ft., before proceeding to higher altitudes. Dr. Raymond Greene informs me that it is not easy to find a place above 14,000 ft. where an expedition could remain long in comfort.

Some animals exhibit tetany under 10 p.c. oxygen, but serum calcium is normal.

Under 10 p.c. the problem is pathological, not physiological.

I am indebted to my colleagues, Dr. J. R. Perdrau and Mr. T. A. Webster, for some occasional help, and also to my technical assistant, Mr. C. Pergande, for much help throughout this research.

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